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U.S. DEPARTMENT OF COMMERCE
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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER
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09/647027

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/01574	March 26, 1999	March 27, 1998

TITLE OF INVENTION
METHOD FOR DETECTING CHANGES IN GENE EXPRESSION LEVEL IN CELLS THAT HAVE BEEN TREATED WITH TEST COMPOUND

APPLICANT(S) FOR DO/EO/US
Masaaki Muramatsu, Hiroshi Wakao, Rika Wakao, Kazuhiro Yano, Teruhisa Noguchi and Akira Suyama

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:
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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1 10 on the date indicated above and is addressed to the Assistant Commissioner For Patents, Washington, D.C 20231

Samantha Bell

Samantha Bell

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17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search report has been prepared by the EPO or JPO $840	$840.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) .. $670	$0.00	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).... $690	$0.00	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $970	$0.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2) to (4) $96	$0.00	
ENTER APPROPRIATE BASIC FEE AMOUNT	$840.00	
Surcharge of $130 for furnishing the oath or declaration later than ☐ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(e)).	$0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	18 - 20	0	x $18	$0.00	
Independent Claims	3 - 3	0	x $78	$0.00	
Multiple Dependent Claims(s) (if applicable)			+ $260	$260.00	
TOTAL OF ABOVE CALCULATIONS				$1,100.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28.)				$0.00	
SUBTOTAL				$1,100.00	
Processing fee of $130 for furnishing the English Translation later than ☐ 20 ☐ 30 mos. from the earliest claimed priority date (37 CFR 1.492(f))				$0.00	
TOTAL NATIONAL FEE				$1,100.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).				$0.00	
TOTAL FEES ENCLOSED				$1,100.00	
				Amount to be refunded	
				Charged	

a. ☒ A check in the amount of $1,100.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Janis K. Fraser, Ph.D., J.D.
FISH & RICHARDSON P.C.
225 Franklin Street
Boston, MA 02110-2804
(617) 542-5070 *phone*
(617) 542-8906 *facsimile*

SIGNATURE — Anita L. Meiklejohn Reg No 35,283

NAME — Janis K. Fraser, Ph.D., J.D.

REGISTRATION NUMBER — 34,819

SPECIFICATION

METHOD FOR DETECTING CHANGES IN GENE EXPRESSION LEVEL IN CELLS THAT HAVE BEEN TREATED WITH TEST COMPOUND

Technical Field

The present invention relates to a method for detecting changes in gene expression levelin cells that have been treated with a compound, a method for screening a gene whose expression level is varied in cells that have been treated with a compound, and a method for screening a compound that alters the expression level of a gene.

Background Art

Drug screening has previously been carried out based on the indicator of whether the drug induces or inhibits a cytobiological event such as cell proliferation, cell survival, cell differentiation, or apoptosis. Most of the cytobiological events secondarily result from induction or inhibition of gene expression, and therefore what gene is affected by treatment with a particular compound is important information on cell fate determination. Detecting changes in the gene expression level induced by the presence of a low-molecular-weight compound, has been used to evaluate the cytotoxicity as well as the cytobiological effect of the compound.

Previously developed methods for detecting differences in gene expression levels between cells (or tissues) at different stages, are differential hybridization (Gene 145: 313-314 (1994) Cloning and sequence analysis of the human SNAP25 cDNA. N. Zhao, H. Hashida, N. Takahashi & Y. Sakaki) and differential display (DD) (FEBS Lett 351: 231-236 (1994) Fluorescent differential display: arbitrarily primed. RT-PCR fingerprinting on an automated DNA sequencer. T. Ito, K. Kito, N. Adati, Y. Mitsui, H. Hagiwara & Y. Sakaki). These methods have been used for detecting changes in gene expression levels among cells at different stages of development, among cells in different phases of cell cycle, between a wild-type cell and a cell lacking a particular gene, or between a normal tissue and a diseased tissue.

In these cases, however, the detection methods are employed

to compare the expression levels of a gene that is expressed in cells without its natural constitution being altered; there is no precedent of detecting changes in the gene expression level in cells that have been artificially treated with a particular compound.

Disclosure of the Invention

An object of the present invention is to provide a method for simply and efficiently detecting changes in gene expression level in cells that have been treated with a compound, a method for simply and efficiently screening a gene whose expression level is varied in cells that have been treated with a compound, and a method for simply and efficiently screening a compound that change the expression level of a gene.

The present inventors studied in order to attain the above-mentioned object, and eventually found that it is possible to detect gene expression level in cells that have been treated with a particular compound, by extracting mRNAs from cells treated with a particular compound and untreated cells (control) and comparing mRNA constitutions. Noting a method in which fluorescence is used as an indicator for detecting differences in the expression level of a particular gene among different types of cells (Nature Biotechnology 14:1675-1680(1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. David J. Lockhart, Helin Dong, Michael C. Byrne, Maximillian T. Follettie, Michael V. Gallo, Mark S. Chee, Michael Mittmann, Chunwei Wang, Michiko Kobayashi, Heidi Horton, and Eugene L. Brown), we investigated how best to apply this method to the detection of changes in gene expression levels. We labeled the cDNAs obtained from the isolated mRNAs, respectively, with different fluorescent labels, mixed them in an appropriate proportion to hybridize them to a particular probe, and then detected the fluorescence from the cDNA hybridized to the probe; the color of fluorescence was varied depending upon the amount of cDNA hybridizing to the probe. The present inventors have thus found that it is possible to simply and efficiently detect changes in gene expression level in cells that have been treated with a particular compound. We have also found that this detection method

makes it possible to screen genes whose expression levels are varied in cells that have been treated with a particular compound and to screen chemical compounds that alter the expression level of a particular gene.

Specifically, the present invention relates to a method, utilizing fluorescent color change, for detecting changes in gene expression level in cells that have been treated with a chemical compound, and also relates to a method, utilizing the above detection method, for screening a gene whose expression level in cells is varied by treating the cells with a particular compound as well as a method, utilizing the detection method, for screening a chemical compound that alters the expression level of a particular gene, and more specifically relates to:

(1) a method for detecting changes in gene expression level in cells that have been treated with a particular compound, the method comprising the steps of:

(a) isolating mRNA from cells treated with the particular compound and mRNA from untreated cells;

(b) obtaining the respective set of cDNAs by reverse transcribing each mRNA isolated;

(c) labeling the respective cDNA sets with different fluorescent labels;

(d) hybridizing each set of labeled cDNAs to a particular probe DNA; and

(e) detecting differences in the amount of cDNAs hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNAs hybridized with the particular DNA probe;

(2) the method as described in (1), wherein the labeled cDNAs are simultaneously hybridized to a large number of probe DNAs;

(3) the method as described in (1), wherein one set of cDNAs is labeled with rhodamine, and the other set is labeled with FITC;

(4) a method for screening a gene whose expression level is varied in cells that have been treated with a particular compound, the method comprising the steps of:

(a) isolating mRNA from cells treated with the particular

compound and mRNA from untreated cells;

(b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated;

(c) labeling the respective cDNA sets with different fluorescent labels;

(d) hybridizing each set of labeled cDNAs to a particular probe DNA;

(e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe ; and

(f) selecting a probe DNA that made the amount of the cDNAs hybridized with the probe DNA different to select the gene corresponding to the probe DNA selected;

(5) the method as described in (4), wherein the labeled cDNAs are simultaneously hybridized to a large number of probe DNAs;

(6) the method as described in (4), wherein one set of cDNAs is labeled with rhodamine, and the other set is labeled with FITC;

(7) a gene whose expression level is varied in cells that have been treated with a particular compound, which is detected by the method as described in any one of (4) to (6);

(8) a vector containing the gene as described in (7);

(9) a transformant carrying the vector as described in (8);

(10) a protein or peptide having an amino acid sequence encoded by the gene as described in (7);

(11) a method for screening a compound that alters the expression level of a gene corresponding to a probe DNA, the method comprising the steps of:

(a) isolating mRNA from cells treated with a test compound and mRNA from untreated cells;

(b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated;

(c) labeling the respective cDNA sets with different fluorescent labels;

(d) hybridizing each set of labeled cDNAs to a particular probe DNA;

(e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe; and

(f) selecting a test compound that made the amount of the hybridized cDNA different;

(12) the method as described in (11), wherein the labeled cDNAs are simultaneously hybridized to a large number of probe DNAs;

(13) the method as described in (11), wherein one set of cDNAs is labeled with rhodamine, and the other set is labeled with FITC; and

(14) a compound that changes the expression level of a particular gene, which is detected by the method as described in any one of (11) to (13).

The present invention relates to a method for detecting changes in gene expression level in cells treated with a particular compound, the method comprising the steps of: (a) isolating mRNA from cells treated with the particular compound and mRNA from untreated cells; (b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated; (c) labeling the respective cDNA sets with different fluorescent labels; (d) hybridizing each set of labeled cDNAs to a particular probe DNA; and (e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe.

In the detection method of the present invention, first, mRNA from cells treated with a particular compound and "mRNA from untreated cells are isolated. There is no specific limitation of the compounds used in the detection method of the present invention; it is possible to use a target compound whose action to change the expression level of a gene is desired to be detected. The compound may be a naturally occurring substance or an artificially synthesized substance.

There is no specific limitation of the cells, which are treated with the compound; it is possible to use a variety of animal or plant cells as well as microbial cells. Preferred examples of the animal cells include 3T3L1 cell, COS cell, NT-2 cell, and Ba/F3 cell, etc. The cells to be treated with a compound in the present invention

include not only cultured cells but also cells inside the body of an animal or plant. The treatment of cells with a compound can be achieved, by an appropriate procedure, for example, by adding the compound to the medium where cells of an animal, plant, or microorganism are cultivated. When the cells are inside the body of an animal, the compound can be given to the animal by a method known to those skilled in the art, for example, by intravascular, intraperitoneal, intraventricular, or oral administration.

It is possible to isolate mRNA expressed in cells treated with a compound by using a method known to those skilled in the art, for example, the guanidine method or a method with oligo(dT) column (Current protocols in Molecular Biology, edit. Ausubel et al. (1987) Publish. John Wiley & Sons).

The mRNAs, isolated from cells treated with the test compound and from untreated cells as a control, are reverse transcribed into the respective sets of cDNAs. Reverse transcription of mRNA is usually carried out by using the whole mRNA isolated from cells, but it is possible, if required, to use mRNA fractionated by a method selected from a variety of separation methods. Reverse transcription of mRNA can be achieved by a method such as SuperScript method using oligo(dT) as a primer (Current protocols in Molecular Biology edit. Ausubel et al. (1987) Publish. John Wiley & Sons).

Subsequently, the sets of cDNAs are respectively labeled with different fluorescent labels. There is no specific restriction of the fluorescent label; it is possible to use rhodamine, FITC, Texas Red, Cy2, Cy3, Cy5, Cy7, or the like. The cDNA can be labeled with fluorescent label by using a method such as nick translation (for example, a commercial kit available from GIBCO-BRL), DNA labeling with random primer (for example, a commercial kit available from GIBCO-BRL), or a method using reverse transcriptase by which the label is directly incorporated into the cDNA using mRNA as a template (Schena, M. et al. (1995) Science 270, 467-470).

In the next step, the respective sets of the labeled cDNAs are hybridized to a particular probe DNA. There is no specific restriction of the particular probe DNA to be used; it is possible to use a probe DNA corresponding to a target gene whose expression

in cells is to be detected after the cells are treated with a compound. Hybridization of a set of the labeled cDNAs to a particular probe DNA can be achieved by a method known to those skilled in the art (see, for example, "Current protocols in Molecular Biology edit. Ausubel et al. (1987) Publish. John Wiley & Sons" and "Schena, M. et al. (1995) Science 270, 467-470").

Subsequently, the difference in the amount of cDNA hybridized to the particular probe DNA is detected among each set of labeled cDNAs based on the color of fluorescent light emitted from the cDNA hybridized to the particular probe DNA. For example, when the particular probe DNA is exposed to a mixture of two sets of cDNAs, one of which labeled with rhodamine and the other labeled with FITC, the cDNAs hybridized with the particular probe DNA emit yellow fluorescence if containing rhodamine-labeled cDNA and FITC-labeled cDNA in equal proportions; green fluorescence if containing FITC-labeled cDNA more abundant than rhodamine-labeled cDNA; and red fluorescence if containing rhodamine-labeled cDNA more abundant than FITC-labeled cDNA. Thus, it is possible to simply detect the difference (abundance) in the amount of DNAs hybridized to the particular probe DNA by detecting the color of fluorescence. The fluorescent color can be detected, for example, by using a scanning laser confocal microscope (type 1X70; Olympus Optical Co., Ltd.), FMBIO (Hitachi Software Engineering Co., Ltd.), or the like.

It is also possible to use a large number of probe DNAs in the detection method of the present invention. In this case, changes in expression levels of many genes, which depend on treatment with a compound, are simply and efficiently detected by using a chip in which a large number of probe DNAs are immobilized on a substrate (hereinafter referred to as genome chip) (see US Patent No. 5,405,783). In the above-mentioned detection method based on the fluorescent color, for example, hybridization is performed to a large number of probe DNA immobilized on a glass substrate of genome chip, and the fluorescence intensity of cDNA hybridized to each of the probe DNAs is measured in a scanning laser microscope, thereby simultaneously detecting the amounts of a large number of different cDNAs. The detection method with genome chip is advantageous in that the cost

is low as well as in that the same operation can be simply repeated.

When the fluorescent color indicating the difference in cDNA amount is observed by the above-described detection method of the present invention, it is possible to screen the gene whose expression level is varied in cells, by choosing a probe DNA corresponding to the cDNA and by selecting the gene corresponding to the probe DNA. Specifically, the present invention also relates to a method for screening a gene whose expression level is varied in cells by treating the cells with a particular compound, the method comprising the steps of: (a) isolating mRNA from cells treated with the particular compound and mRNA from untreated cells; (b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated; (c) labeling the respective cDNA sets with different fluorescent labels; (d) hybridizing each set of labeled cDNAs to a particular probe DNA; (e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe; and (f) selecting a probe DNA that made the amount of cDNA hybridized with the probe DNA different to select the gene corresponding to the probe DNA selected.

A target gene (for example, a gene whose expression level is elevated beyond a fixed value or decreased below a fixed value) is selected by this screening method of the present invention, from genes whose expression levels are detected to vary by the inventive detection method. A selected gene, when hybridizing to a probe of a known DNA, can be identified as a gene having the same nucleotide sequence as that of the probe DNA or as a gene having a similar nucleotide sequence to that. Once the target gene whose expression level is varied in cells by treating the cells with a compound, is isolated by the screening method of the present invention, the protein encoded by the gene can be prepared by introducing the gene inserted in an appropriate vector into a host cell and extracting the protein from the transformant. The host cell into which the vector is introduced can be, for example, any of various animal cells (for example, COS cell, etc.), *E. coli*, yeast, and insect cells or others. The vectors used preferably are "pME18S vector" (Mol Cell Biol 8:

466-72 (1988) SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Y. Takebe, M. Seiki, J. Fujisawa, P. Hoy, K. Yokota, K. Arai, M. Yoshida & N. Arai) for COS cell and NR-2 cell; "pET vector" (Takara shuzo) for *E. coli*, and BacPac (Clontech) vector for insect cells. A vector can be introduced into a host cell according to a usual method, for example, electroporation for *E. coli*, lipofectamine (GIBCO-BRL) method for COS cell, or a method using Baculovirus infection for insect cells. A recombinant protein can be purified from transformants expressing the protein by a combination of usual methods including various types of chromatography, electrophoresis, and gel filtration, in accordance with properties of the target protein. Column purification methods such as His-tag method and HA-tag methods are also usable.

When the fluorescence color indicating the difference in cDNA amount is detected by the above-described detection method of the present invention, it is also possible to screen compounds that alter the expression level of a particular gene in cells, by selecting a test compound used for the treatment of the cells from which the cDNA isolated. Specifically, the present invention further relates to a method for screening a compound altering the expression level of a gene corresponding to a probe DNA, the method comprising the steps of: (a) isolating mRNA from cells treated with a test compound and mRNA from untreated cells; (b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated; (c) labeling the respective cDNA sets with different fluorescent labels; (d) hybridizing each set of labeled cDNAs to a particular probe DNA; (e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe; and (f) selecting a test compound that made the amount of the hybridized cDNA different. A compound library, for example, is usable as a compound for the treatment of cells in this screening method. The compound library can be prepared according to a method known to those skilled in the art (for example, Application of Combinatorial Library

Methods in Cancer Research and Drug Discovery. Lann, K. S. (1997) Anticancer Drug Des 12: 145-167). In this screening method, for example, when a probe DNA of a gene associated with a disease is used as the probe DNA for detecting changes in expression level of a particular gene, the compound isolated is a candidate for a drug for treating the disease.

Brief Description of the Drawings

Figure 1 shows a result of detection of genes whose expression is induced by retinoic acid and FCS in 3T3L1 cells. Forty eight clones obtained by differential screening, which are believed to be specific to 3T3L1 cells that had been treated with retinoic acid and FCS, were immobilized in duplicate onto nylon membranes. In each group of A to H, same clones as each other are 1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10, or 11 and 12.

Best Mode for Carrying out the Invention

The present invention will be described more specifically below with reference to Examples, but is not to be construed as being limited thereto.

[Example 1] Detection of genes whose expression is induced in 3T3L1 cells

3T3L1 cells (passaged in DMEM containing 10% FCS), grown to be confluent, were incubated for 18 hours in serum-free DMEM, and then the cells were divided into two groups, A and B. One-micromole/litter retinoic acid (RA) and 10% FCS were added into the medium of the group B cells, but not into that of the group A. Both groups were further incubated for 3 hours. Messenger RNA was extracted from each of the group A and group B cells by using mRNA Isolation System (GIBCO-BRL). The isolated mRNAs were reverse transcribed into first strand cDNA by using OligodT(12) as a primer, SuperScript II (GIBCO-BRL), and the attached buffer. The resulting first strand cDNAs were used as templates to synthesize DIG-11-dUTP-labeled cDNAs. The labeled cDNAs (A) and (B) were prepared by using a DIG labeling & detection kit (Boehringer Mannheim).

The two cDNA libraries of 3T3L1 cell, in which one had been treated with 1μM retinoic acid (RA) and 10% FCS but the other had not, were subjected to differential screening; 48 positive clones (cDNAs assumed to be specific to the cDNA library of 3T3L1 cells treated with 1μM retinoic acid (RA) and 10% FCS) were selected and isolated. The DNAs were spotted by 1.5-mm intervals in duplicate onto a nylon membrane and then UV-crosslinked. This membrane was probed with the labeled cDNAs (A) and (B) in a hybridization buffer, HybSeq (Stratagene); hybridization was performed at 42℃ for 2 hours. The membrane was washed with 2xSSC (twice at room temperature and then twice at 42℃), and further washed once with 0.2xSSC at 68℃. The signal was visualized on Kodak X-OMat. The result showed that, as shown in Figure 1, there were several genes (A34, A56, B12, B34, B56, and F56) whose expression was induced by retinoic acid and FCS in 3T3L1 cells.

[Example 2] Preparation of a chip by immobilizing DNA on a substrate

As described below, the oligo DNAs (Table 1) of sequences 1 to 6 (the respective nucleotide sequences are indicated in SEQ ID NOs: 1 to 6) were immobilized on a glass substrate by photolithography (Hermanson, G. T., et al., "Immobilized Affinity Ligand Techniques", Academic Press, Inc., San Diego, CA (1992); Robertson, S. A., et al., J. Am. Chem. Soc., 113, 2722-2729 (1991)).

Table 1

Sequence 1: 5′-AGCAGCAGCAACGAGCCCTCCTCCGACTCCCTGAGCTCACCCACGCTGC TGGCCCTGTGA--3′

Sequence 2: 5′-CTCCGACTCCCTGAGCTCACCCACGCTGCTGGCCCTGTGA--3′

Sequence 3: 5′-CCACGCTGCTGGCCCTGTGA--3′

Sequence 4: 5′-TGGCTCCATCCTGGCCTCACTGTCCACCTTCCAGCAGATGTGGATCAGC AAGCAGGAGTA-3′

Sequence 5: 5′-TGTCCACCTTCCAGCAGATGTGGATCAGCAAGCAGGAGTA-3′

Sequence 6: 5′-TGGATCAGCAAGCAGGAGTA-3′

Specifically, the surface of a glass slide (MATSUNAMI, S0313) was treated with 0.05M HCl for 1 hour, washed several times with ultra

pure water, and then the slide was immersed into ethanol. Subsequently, the glass slide was immersed into an A solution (diethyl ether:toluene = 1:1), B solution (toluene), and C solution (toluene:3-(2-aminoethylaminopropyl)trimethoxy silane = 10:1) in this order, the slide immersed in the C solution was placed as a whole with the container in a desiccator, which is ventilated with nitrogen, then the slide was aminosilanated overnight. Subsequently, the slide was washed several times with ethanol and then with ultra pure water for 2 hours. The slide was then dried under vacuum in a desiccator (see, as a reference, Hermanson, G. T., et al., Academic Press, Inc., San Diego, California (1992)). A capping reagent (4,5-dimethoxy-2-nitrobenzyl chloroformate) of 10 mg/ml (dissolved in THF (tetrahydrofuran)) was placed on the silanated glass slide. After reacted for 10 minutes, the slide was washed with THF and then with ultra pure water, and was dried with nitrogen gas (see, as a reference, Robertson, S. A. et al., J. Am. Chem. Soc. 113, 2722-2729 (1991). Potassium acetate (1 mM, pH 4.5) was loaded on positions to be decapped on the slide. Under a microscope, the slide was subjected to excitation light irradiation with a mercury lamp (with a 330-380 filter) for 30 minutes to give rise to a decapping reaction. After the reaction, the slide was washed several times with ultra pure water. The slide was covered with 10 mM DSS (disuccinimidyl suberate) and allowed to react for 10 minutes, then the slide was washed, and flushed with nitrogen gas to dryness. Next, 0.2 μl of oligo DNA (100 μM) was placed on the surface and allowed to react for 3 minutes. The slide was then rinsed several times with TE (Tris-HCl (pH 7.5)/1 mM EDTA) to remove unreacted NHS groups, washed with ultra pure water, and dried with nitrogen gas.

The nucleotides shown in SEQ ID NOs: 1 to 3 are to be used as probes for detecting the c-fos gene (Cell 32: 1241-55 (1983) [MUID: 83180421] Analysis of FBJ-MuSV provirus and c-fos (mouse) gene reveals that viral and cellular fos gene products have different carboxy termini. C. Van Beveren, F. van Straaten, T. Curran, R. Muller & I. M. Verma). The nucleotides shown in SEQ ID NOs: 4 to 6 are to be used as probes for detecting the β-actin (J Mol Evol 23: 11-22 (1986) [MUID: 86200234] Comparison of three actin-coding sequences in the mouse;

evolutionary relationships between the actin genes of warm-blooded vertebrates. S. Alonso, A. Minty, Y. Bourlet & M. Buckingham).

[Example 3] mRNA preparation from cultured cells and synthesis of fluorescence-labeled cDNA

12-o-tetradecanoylphorbol-13-acetate (TPA, 1μM), or DMSO as a control, was added to a culture medium of Ba/F3 cells (the cells treated with the respective reagents are denoted as (a) group and (b) group). After 30 minutes, the cells were harvested, and mRNA was prepared from the cells by using a "Quick prep micro mRNA purification kit" (Pharmacia). Concentrations of mRNAs obtained from (a)- and (b)-group cells were determined based on their OD values and then normalized. The cDNAs were prepared from the mRNAs by reverse transcription with a "cDNA first strand kit" (Pharmacia). In the reaction, the cDNA derived from the (a) group was fluorescence-labeled by incorporating "Cy5-dCTP" (Biological detection systems) into the cDNA. The cDNA derived from the (b) group was fluorescence-labeled by incorporating "FluorX-dCTP" (Biological detection systems) into the cDNA.

[Example 4] Hybridization of fluorescence-labeled cDNA to synthetic DNA on a glass substrate

A mixture containing equal amounts of the fluorescence-labeled cDNAs derived from the (a) and (b) group samples was hybridized to the synthetic DNA on the glass slide. Hybridization was carried out with a solution of 6XSSPE containing 0.5 mg/ml salmon sperm DNA and the fluorescence-labeled cDNA mixture at 50℃ for 16 hours. The slide was then washed with 6xSSPE (at 25℃) and with 0.5xSSPE (at 50℃).

[Example 5] Detection of the fluorescence-labeled hybridized cDNA

The fluorescence-labeled cDNA was detected using a glass plate reader. The reader is composed of a down-illuminating fluorescence microscope (Olympus Optical Co., Ltd.) combined with a glass slide holder and a SIT high-sensitive detection camera (Hamamatsu Photonics K.K.). The result showed that a greenish interference wavelength emitted from FluorX, was observed at each spot to which SEQ ID NO:

1, 2, and 3, probes for detecting the c-fos gene, were bound. On the other hand, a yellowish interference wavelength emitted from FluorX and Cy5, was observed at each spot to which SEQ ID NO: 4, 5, and 6, probes for detecting the β-actin gene, were bound. These results suggest that c-fos mRNA is significantly abundant in the (a) group sample whereas amounts of β-actin mRNA are comparable to each other in the samples of (a) and (b) groups.

Industrial Applicability

The present invention provides a method for detecting changes in gene expression level in cells, which is varied by treating the cells with a compound, a method for screening genes whose expression levels are varied in cells treated with a compound, and a method for screening compounds that alter the expression level of a gene. By these methods, when respective sets of cDNAs, which are used for detecting changes in gene expression level, are labeled with different fluorescent labels, it is possible to simply and efficiently detect changes in gene expression level by using the florescent color as an indicator. Furthermore, changes in gene expression level can be efficiently detected by using a genome chip on which a large number of probe DNAs are immobilized.

CLAIMS

1. A method for detecting changes in gene expression level in cells treated with a particular compound, the method comprising the steps of:

(a) isolating mRNA from cells treated with the particular compound and mRNA from untreated cells;

(b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated;

(c) labeling the respective cDNA sets with different fluorescent labels;

(d) hybridizing each set of labeled cDNAs to a particular probe DNA; and

(e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe.

2. The method of claim 1, wherein the labeled cDNAs are simultaneously hybridized to a large number of probe DNAs.

3. The method of claim 1, wherein one set of cDNAs is labeled with rhodamine, and the other set is labeled with FITC.

4. A method for screening a gene whose expression level is varied in cells by treating the cells with a particular compound, the method comprising the steps of:

(a) isolating mRNA from cells treated with the particular compound and mRNA from untreated cells;

(b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated;

(c) labeling the respective cDNA sets with different fluorescent labels;

(d) hybridizing each set of labeled cDNAs to a particular probe DNA;

(e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe; and

(f) selecting a probe DNA that made the amount of cDNA hybridized with the probe DNA different to select the gene corresponding to the probe DNA selected.

5. The method of claim 4, wherein the labeled cDNAs are simultaneously hybridized to a large number of probe DNAs.

6. The method of claim 4, wherein one set of cDNAs is labeled with rhodamine, and the other set is labeled with FITC.

7. A gene whose expression level is varied in cells by treating the cells with a particular compound, which is detected by the method of any one of claims 4 to 6.

8. A vector containing the gene of claim 7.

9. A transformant carrying the vector of claim 8.

10. A protein or peptide having an amino acid sequence encoded by the gene of claim 7.

11. A method for screening a compound that alters the expression level of a gene corresponding to a probe DNA, the method comprising the steps of:

(a) isolating mRNA from cells treated with a test compound and mRNA from untreated cells;

(b) obtaining a set of cDNAs by reverse transcribing each mRNA isolated;

(c) labeling the respective cDNA sets with different fluorescent labels;

(d) hybridizing each set of labeled cDNAs to a particular probe DNA;

(e) detecting differences in the amount of cDNA hybridized with the particular DNA probe among each set of the labeled cDNAs in terms of the color of fluorescence emitted from the cDNA hybridized with the particular DNA probe; and

(f) selecting a test compound that made the amount of cDNA hybridized different.

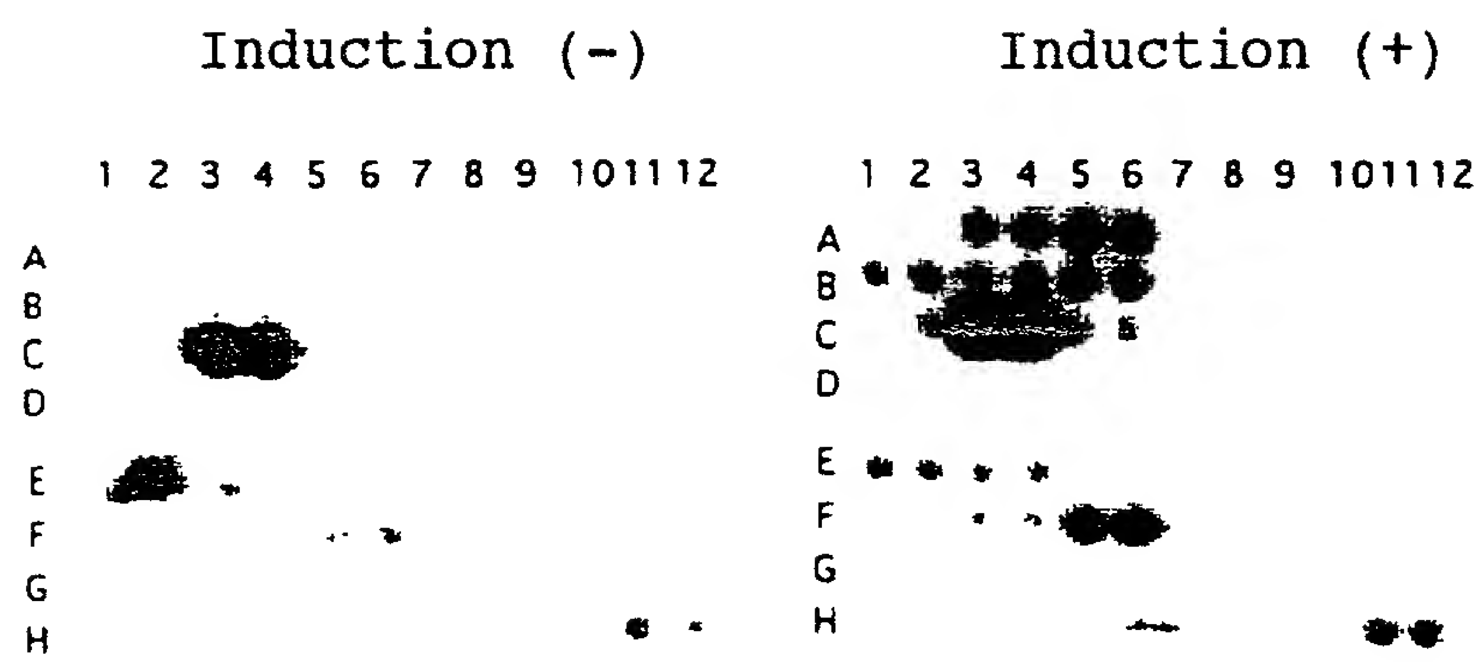
12. The method of claim 11, wherein the labeled cDNAs are simultaneously hybridized to a large number of probe DNAs.

13. The method of claim 11, wherein one set of cDNAs is labeled with rhodamine, and the other set is labeled with FITC.

14. A compound that alters the expression level of a particular gene,

which is detected by the method of any one of claims 11 to 13.

Figure 1



COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled METHOD FOR DETECTING CHANGES IN GENE EXPRESSION LEVEL IN CELLS THAT HAVE BEEN TREATED WITH TEST COMPOUND, the specification of which:

[] is attached hereto.
[X] was filed on September 26, 2000 as Application Serial No. 09/647,027 and was amended on _____.
[] was described and claimed in PCT International Application No. _____ filed on _____ and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
Japan	10/100096	March 27, 1998	[X] Yes [] No
PCT	PCT/JP99/01574	March 26, 1999	[X] Yes [] No

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Janis K. Fraser, Reg. No. 34,819
Timothy A. French, Reg. No. 30,175
Ralph A. Mittelberger, Reg. No. 33,195
John W. Freeman, Reg. No. 29,066
Anita L. Meiklejohn, Reg. No. 35,283
J. Peter Fasse, Reg. No. 32,983

Address all telephone calls to JANIS K. FRASER at telephone number (617) 542-5070.

Address all correspondence to JANIS K. FRASER at:

FISH & RICHARDSON P.C.
225 Franklin Street
Boston, MA 02110-2804

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Combined Declaration and Power of Attorney

100

Full Name of Inventor: MASAAKI MURAMATSU

Inventor's Signature: Masaaki Muramatsu Date: Nov. 14, 2000

Residence Address: 3-15-13, Koyama, Nerima-Ku
TOKYO 176-0022 JAPAN JPX

Citizenship: Japan

Post Office Address: 3-15-13, Koyama, Nerima-Ku
TOKYO 176-0022 JAPAN

200

Full Name of Inventor: HIROSHI WAKAO

Inventor's Signature: Hiroshi Wakao Date: Nov. 14, 2000

Residence Address: 5-29-6, Hachimandai, Kisarazu-shi
CHIBA 292-0814 JAPAN JPX

Citizenship: Japan

Post Office Address: 5-29-6, Hachimandai, Kisarazu-shi
CHIBA 292-0814 JAPAN

300

Full Name of Inventor: RIKA WAKAO

Inventor's Signature: Rika Wakao Date: Nov 14, 2000

Residence Address: 5-29-6, Hachimandai, Kisarazu-shi
CHIBA 292-0814 JAPAN JPX

Citizenship: Japan

Post Office Address: 5-29-6, Hachimandai, Kisarazu-shi
CHIBA 292-0814 JAPAN

Combined Declaration and Power of Attorney

400

Full Name of Inventor: KAZUHIRO YANO

Inventor's Signature: Kazuhiro Yano Date: 20/11/2000

Residence Address: 1-19-11, Nagaomotomachi, Hirakata-shi
OSAKA 573-0163 JAPAN JPX

Citizenship: Japan

Post Office Address: 1-19-11, Nagaomotomachi, Hirakata-shi
OSAKA 573-0163 JAPAN

500

Full Name of Inventor: TERUHISA NOGUCHI

Inventor's Signature: Teruhisa Noguchi Date: 7/12/2000

Residence Address: 4-11-18-701, Minamiazabu, Minato-ku
TOKYO 106-0047 JAPAN JPX

Citizenship: Japan

Post Office Address: 4-11-18-701, Minamiazabu, Minato-ku
TOKYO 106-0047 JAPAN

600

Full Name of Inventor: AKIRA SUYAMA

Inventor's Signature: Akira Suyama Date: 15/12/2000

Residence Address: 2-6-501, Shimoyugi 3-chome, Hachioji-shi
TOKYO 192-0372 JAPAN JPX

Citizenship: Japan

Post Office Address: 2-6-501, Shimoyugi 3-chome, Hachioji-shi
TOKYO 192-0372 JAPAN

SEQUENCE LISTING

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